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OK to Enter
GREGORY MILLS
QUALITY ASSURANCE SPECIALIST



February 14, 2008

Mr. Keith Hendricks
United States Patent and Trademark Office
Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Re: Patent Nos. 7250184, 6537598 and 6814989

Mr. Hendricks,

Enzyme Development Corporation wishes to protest the three listed patents. We have just become aware of these patents or would have protested earlier. Our basis for the protest is that there is significant prior art regarding the use of various enzymes for meat tenderizing. Papain, bromelain and ficin have been commercially sold for meat tenderizer for many decades. The approvals for all of these enzymes have been listed in the USDA approved food additives regulations for many decades. In addition, bromelain, ficin, and papain were listed on the GRAS Affidavit Petition filed with the FDA in 1972 for enzymes in commercial use prior to 1958.

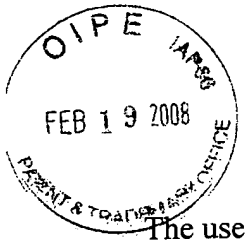
There are many prior patents and published information on the use of enzymes as tenderizers. For example, Enzymes in Food Processing and Products 1972, Wieland, Henry, Noyes Data Corporation has an extensive discussion of meat tenderizing enzymes including blends of the enzymes. Copies of pages 132 to 152 are included with the letter. As an example, patent 3,276,879 specifically mentions blends of bromelain, ficin, and papain.

In the 1980's Enzyme Development Corporation published several trade documents discussing bromelain, papain and ficin as meat tenderizers and, more recently an updated version has been on our website since the inception of the website; approximately eight years ago.

Enzyme Development Corporation has also marketed blends of bromelain, ficin and papain under the trade name "Enzeco® Triple Protease" and "Enzeco® Dual Protease". These products were specific to the tenderizer industry and have been in general commerce for many years.

There are numerous blends of the various enzymes that are sold to the food service industry for meat tenderizing. The enzymes are required by law to be part of the ingredient listing on the various marinade blends and treated meat products.

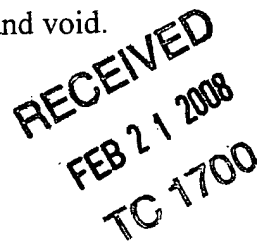
# Enzyme Development Corporation




The use of Actinidin as a meat tenderizer was published in 1988, (abstract enclosed). It is not currently allowed as an approved food additive in the United States. Were the product allowed, it would have been an obvious addition for meat tenderizer.

Our objections to these Patents are based on substantial prior art, commercially established blends of the various enzymes, and the fact that the blend would be "obvious" since all are established meat tenderizer enzymes.

We hope you will re-examine the various patents and declare them null and void.




Respectfully,

ENZYME DEVELOPMENT CORPORATION

C. Peter Moodie
Director of Sales and Marketing

Encs: EDC Website Article, Meat Tenderizing Enzymes, a brief discussion
Pages 132-152 Enzymes in Food Processing and Products

Cite:
MIYADA, D. S. and TAPPEL, A. L. The Hydrolysis of Beef Proteins by Various Proteolytic Enzymes. Food Research 21 217 (1956)

Deborah A. Lewis, B. S. Luh (1988) Application of Actinidin from Kiwifruit to Meat Tenderization and Characterization of Beef Muscle Protein Hydrolysis
Journal of Food Biochemistry 12 (3), 147-158

## Meat Tenderizing Enzymes, a brief discussion

The two most often used meat tenderizing enzymes are Papain and Bromelain. Both are derived from plant sources. These are the papaya fruit and the pineapple plant. To a much lesser extent, Ficin, derived from fig tree latex is also used. Other sources of enzymes have been cited for meat tenderization such as *Bacillus subtilis, Aspergillus oryzae* and even pancreatin derived from the pancreas gland (typically hog).

### PAPAIN

Papain is usually produced as a crude, dried material by collecting the latex from the fruit of the papaya tree. The latex is collected after scoring the neck of the fruit whereupon it may either dry on the fruit or drip into a container. This latex is then further dried. It is now classified as a dried, crude material. A purification step is necessary to remove contaminating substances. This purification consists of the solubilization and extraction of the active papain enzyme system through a government registered process. This purified papain may be supplied as dried powder or as a liquid.



### BROMELAIN

Bromelain is prepared from the stump or root portion of the pineapple plant after harvest of the fruit. This stump or root portion is collected from the fields, peeled and crushed to extract the juice containing the soluble Bromelain enzyme. Further processing includes precipitation of the enzyme to further purify it. This process is carried out in factories under strictly controlled conditions to assure microbiological quality and enzyme purity. The Bromelain products are all supplied as powders. The other enzymes mentioned are produced using selected micro-organisms, such as *Bacillus subtilis* and *Aspergillus oryzae* in commercial enzyme production facilities. Roughly 95 plus percent of the meat tenderizing enzymes consumed in the United States are from the plant proteases - Papain and Bromelain. The microbial tenderizers constitute a minimal portion and have never been successfully applied on a large scale.

### APPLICATIONS

The technical details concerning the various muscle tissue acted upon by the enzymes is discussed in depth in Part VIL Chapter 27, "Applied Enzymology of Meat Texture Optimization" of the book entitled, *Source Book of Food Enzymology*, by Sigmund Schwimmer, Ph.D. There are various opinions and approaches to the process of tenderizing meats. One is the antemortern use of meat tenderizing enzymes. This consists of the physical injection of a controlled solution of either papain or some other enzyme into the living animal. This practice has been discontinued and is no longer used. Postmortem application is generally acceptable for the lesser quality cuts and a variety of application methods are available. Often, the enzyme is included as part of a marinade.

The major area of consumption of meat tenderizers that we see in the United States is in consumer households. This consumer use probably accounts for 90% of enzyme tenderizer sales. Typically two products are being sold in grocery stores ... papain and bromelain.

For this application, the consumer sprinkles the powder containing the standardized enzyme material on the meat and through a mechanical process called "forking" have the enzyme penetrate the meat cut and then immediately cook in order to produce a tenderized and highly palatable product. Some of these types of tenderizers are blended with various spices and flavor enhancers such as monosodium glutamate.

Further refinements of home use are the incorporation of the enzymes in marinades that both flavor and tenderize tough cuts of meat. The major application of tenderizer in today's market is beef. However many interesting approaches are possible for other types of meat such as hams and even chicken from non-prime sources such as old egg laying hens. A newer area is seafood. The products being treated are squid (calamari), clams, and other very tough and chewy seafood.

**CHARACTERISTICS**
The general characteristics of the two plant derived enzymes vary somewhat since they all have different temperatures of inactivation and operate with different kinetics when applied.

Papain is the most temperature stable and can require a temperature as high as 170-185oF to completely inactivate it. This has certain advantages and certain disadvantages. The main disadvantage is that a piece of meat cooked to what we call "medium rare" will not reach a temperature high enough to inactivate the papain. Thus, subsequent storage of the meat will allow the enzyme to continue to tenderize and if extended over too long a period will produce a mushy unpalatable texture. Papain should be used in very controlled processes where each step and cut of meat is under controlled time and temperature and served properly to the consumer. This is the best process for large scale highly organized restaurant chains where the process is thoroughly outlined and adhered to. The pH optimum of papain is typically similar to that of meat itself.

Bromelain has a lower temperature of inactivation and a slightly different mode of operation. The temperature of inactivation of Bromelain is around 160o F which, again, will not be high enough for inactivation in a medium rare piece of beef. The rate of action for both papain and bromelain are similar and, therefore, timing for processing would be similar.

We also supply blends of these products in a ratio which provides, for certain applications, a unique tenderizing effect.

The microbial enzymes such as *Aspergillus oryzae* are not commonly used and we cannot give you much Information concerning their applications in this area. They are, however, mentioned in the Schwimmer book.

**ACTIVITY-POTENCY**
The most important consideration in selecting a tenderizing enzyme is the activity of the enzyme. Further considerations are that the material be of food grade quality, that it have a low microbial count, and that it meets all incidental government specifications. Activity is a measure of the enzyme's ability to react with a specific substrate chosen by the supplier. Enzymes are sold on the basis of activity or potency. One of the most common assays for Papain and Bromelain is the Milk Clot Assay.

The Milk Clot Assay is a very accurate and yet simple test procedure which measures the amount of time required to form clotted milk in the presence of the proteolytic enzyme under specified and controlled conditions, i.e., temperature etc. Using this number, whether it is 100 units per mg. or 500 units per mg., the buyer can immediately assign formulations that will consistently yield the same quality of tenderization during the application.

**DOSE OR APPLICATION RATE**

A Finished Blend for Home Use

Typically enzyme preparations used for direct application by the ultimate consumer are standardized to contain 0.75 to 1.5 MCU (milk clot units) of enzyme activity per mg of finished product. The general application rate of this finished product is 1 teaspoon or 3 grams per pound (500 grams) of meat. This is the type of product that would be sold in the grocery store and applied by the consumer. At 3 grams of blended tenderizer, the consumer would be using a dose of papain calculated as follows:
3 grams = 3000 mg

Formula 1 , standardized at 0.75 MCU/mg

3000 mg x 0.75 MCU/mg enzyme activity = 2250 Milk Clot Units per pound of meat

Formula 2, standardized at 1.5 MCU/mg

3000 mg x 1.5 MCU/mg enzyme activity = 4500 Milk Clot Units per pound of meat

**For Commercial Marinades and Other Food Service Applications**

The action of the enzyme will depend on the time and temperature that the enzyme has to work. As an example, a cut of meat may be injected with a marinade and then vacuum tumbled to finish absorption and forming. The meat is then flash frozen and thawed when ready for use. Depending on a variety of factors, the marinade should be formulated so that one pound of meat receives between 1000 and 3000 Milk Clot Units. As an example, if PANOL® papain, (Activity 300 Milk Clot Units per milligram), were used in the formula, the researcher would start testing at 3.3 mg per pound of meat to be treated and increase the dose up to 10 mg. or until the desired tenderness were achieved.



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Example 3: A third group of live eight week old chickens were tested to demonstrate the spreading effect of hyaluronidase and to demonstrate that a live broiler can be injected 5 minutes before dispatch with a definite enhancement of flavor. After the injection the birds were held for the indicated time interval, dispatched and processed in the customary manner, chilled in ice slush for 16 hours, iced and stored under refrigeration at 40°F. for 24 hours, and cooked in a 350°F. oven.

| Time Lapse Between Injection and Dispatch | Material Injected | Flavor After 350°F. Oven Cook |
|---|---|---|
| 30 minutes | 1 1/2 sage oil and 0.05 cc hyaluronidase | Definite sage flavor |
| 15 minutes | 1 1/2 sage oil and 0.05 cc hyaluronidase | Definite sage flavor |
| 5 minutes | 1 1/2 sage oil and 0.05 cc hyaluronidase | Definite sage flavor |
| 5 minutes | 1 1/2 garlic oil and 0.05 cc hyaluronidase | Definite garlic flavor |
| 5 minutes | 1 1/2 sesame oil and 0.05 cc hyaluronidase | Definite sesame flavor |

## PROTEOLYTIC ENZYME FOR PRESLAUGHTER ENZYME TREATMENT

J.M. Hogan and H.F. Bernholdt; U.S. Patent 3,163,540; December 29, 1964; assigned to Swift & Company have developed a process for the preslaughter enzyme treatment of meat-bearing animals to provide tendered meat cuts derived from such animals. The method includes the steps of rapidly introducing proteolytic enzymes into the living animal's vascular system and promptly slaughtering the animal. It is thus possible to insure nonuniform distribution of the enzyme throughout the vascular system and supply sufficient enzyme to muscle and other tissues to insure that the meat cuts derived from such animals will possess an improved tenderness upon cooking. The incomplete distribution of enzymes insures that lesions in the carcass resulting from enzyme action on certain organs are minimized.

The enzyme introduction step is carried out by any means which insures introduction of the enzyme into the vascular system of the living animal. Poultry are usually injected with a hypodermic syringe into one of the exposed veins such as the humeral or internal metatarsal veins. Flow of solution into the animal can be by gravity or utilizing some pressure to speed up the enzyme introduction step. The concentration of enzyme in solution should be sufficient to insure that the total amount of solution required for a given animal will not necessitate an extended period of time for introduction. Solutions of plant-derived proteolytic enzymes such as papain, ficin and bromelin and mixtures thereof having proteolytic activities of around 500 to 14,000 tyrosyl units/milliliter are satisfactory for this purpose. Such a solution permits introduction of the enzyme into the animal in a quantity of about 0.1 to 0.35 ml./lb. of live animal weight.

Poultry such as large roosters and turkeys, have a circulation time of around 2 to 3 seconds, and it is advisable to complete the enzyme introduction and slaughter as promptly as possible. Very desirable results are obtainable where enzyme introduction and slaughter are completed in not more than about three seconds.

Example: A series of 12 turkeys averaging about 25 to 30 pounds each were injected with

an aqueous solution of papain utilizing enough of the solution to provide 0.1 ml./lb. of a solution having an activity represented by 1,000 tyrosyl units/ml. A conventional syringe was used and injection was into the jugular vein. One group of the turkeys were slaughtered immediately after completion of the injection, another group was slaughtered 5 seconds after completion of the injection, and another group was slaughtered 10 seconds after completion of the injection. Tenderness and texture of light and dark meat was evaluated and the texture of livers was also measured. The benefits resulting from quick slaughter (less than seconds after injection) were apparent.

## ANTEMORTEM TENDERIZATION PROCESS

In the process of J.M. Hogan; U.S. Patent 3,235,468; February 15, 1966; assigned to Swift & Company proteolytic enzymes are introduced into living animals to obtain uniform distribution of the enzymes throughout the meat tissues. In U.S. Patent 2,903,362, a method of treating living animals from which meat is to be obtained with proteolytic enzymes to provide, after slaughtering, carcasses and meat cuts which have improved tenderness when cooked. These qualities are attributed to the substantially uniform distribution of the proteolytic enzyme in the meat tissues.

While a satisfactory method for preparing enzymes in a form suitable for use in this process is given in the patent, and the meat products produced possess the desired improved tenderness and texture, further and additional improvements upon this process have been developed. These improvements allow for standardization of the enzyme used in the process and control of the tenderizing action by pretreatment of the enzyme before introduction into the animal and, also, adjustment of the enzymatic activity and control of the amount of a given enzyme of a predetermined activity which is introduced into the animal.

One of the types of enzyme used in this process are those proteolytic enzymes derived from plant sources. It is known that proteolytic enzymes derived from plant materials are of variable constitution. Variations in constitution of the enzyme are also introduced in the handling of the enzyme during processing so that the protein content, proteolytic activity, color, odor, contamination, etc., will vary widely depending upon the source of the enzyme and the processing to which it has been subjected.

Crude papain, ficin, and bromelin contain a certain amount of enzymic materials which are active at temperatures in the range normally used in cooking meat products and, also, certain enzymic materials which are active at lower temperature and which may adversely affect the animal body processes. Satisfactory tenderization with a minimum of adverse physiological reactions in the animal is desired in the antemortem procedure. However, the degree of tenderization and the avoidance of physiological reactions is determined to a significant degree by the properties of the enzyme employed.

The process is involved with the production of enzyme materials which have a high proteolytic activity and a reduced tendency to adversely affect meat-bearing animals into which they are introduced. The enzymes are especially adapted for use in the tenderization of meats since they exhibit a high degree of proteolytic activity at normal cooking temperatures.

Coupled with the high proteolytic activity in the range of 140° to 210°F. is a minimum amount of proteolytic activity at temperatures below this range and specifically in the range around normal animal body temperature.

The enzyme generally is derived from plant sources and possesses a proteolytic activity expressed as tyrosyl units/g. of 40,000 to 200,000 tyrosyl units. In solution the enzyme should have an activity expressed in tyrosyl units/ml. of at least 250 and preferably 500 tyrosyl units and a ratio of tyrosyl units/ml. to total milk clotting units/ml. of 55 to 100, as well as demonstrating a satisfactory tissue specificity assay, at a temperature in the range of 140° to 210°F. Another characteristic of the enzyme solutions is that activity is also represented by a ratio of tyrosyl units/ml. to available milk clotting units/ml. of more than 200 and preferably more than 333.

The activity of a proteolytic enzyme as represented by tryosyl units provides a measure of the tenderizing effect of the enzyme. Since this determination is carried out at an elevated temperature, it measures potential tenderizing effect at these elevated temperatures. Milk clotting units is an expression of activity of the enzyme at 40°C. and is a good measure of activity at or near the animal's body temperature. Thus, it is a good measure of the likelihood of adverse physiological reactions presented by enzymic activity at body temperature. Accordingly, the activity represented by tyrosyl units ideally should be high and activity represented by milk clotting units, both total and available, should be low in relation to the tyrosyl units. The ratio of activity expressed as tyrosyl units to the activity expressed as total milk clot units and available milk clot units provides an evaluation of the maat tenderizing activity of the enzyme and, also, provides a measure of the likelihood of the enzyme composition to adversely affect the physiological processes of the animal.

Tyrosyl units represent the increased quantity of trichloracetic soluble compounds capable of producing color with the phenol color reagent equivalent to that produced by 100 micrograms of tyrosine, derived from 1 g. of meat, when the enzyme is incubated with meat for 1 hour at 80°C. under specified condition. Thus, the rating of an enzyme composition in tyrosyl units is a measure of the proteolytic activity of the enzyme on meat at or about 80°C. Although enzyme compositions having a considerable range of tyrosyl units/ml. can be used in the process, very dilute or extremely concentrated solutions are not generally recommended.

Very dilute solutions containing enzyme in an amount sufficient to provide an activity represented by only about 100 tyrosyl units/ml. require that excessively large volumes be introduced into the animal to obtain any measurable tendering effect. Where very concentrated preparations where enzyme activity is around 10,000 tyrosyl units/ml. are used care must be employed in the metering and measuring of solution and careful control of the time between completion of the injection and slaughter must be maintained to avoid adverse reactions in the animal. Such concentrated solutions preferably should be diluted prior to use.

For best results the enzyme is suspended in water in a concentration sufficient to provide an activity of about 1,000 tyrosyl units/ml. This permits introduction of the solution into the animal in a quantity of 0.1 to 0.35 ml./lb. of live animal weight. For the average animal in the range of 800 to 1,100 lbs. the total volume of enzyme solution required is then in the range of 80 to 385 ml. and this amount can be injected in 16 to 77 seconds. More dilute

solutions having only 500 tyrosyl units/ml. must be introduced at the rate of 0.2 to 0.7 ml. per pound and with the average animal this requires 160 to 770 ml. total injection. More concentrated solutions having about 5,000 tyrosyl units/ml. require that only 0.02 to 0.07 ml./lb. be introduced and the total amount is only 16 to 77 ml. which can be injected in 3 to 16 seconds although with these more concentrated solutions greater precision in the injection step is advisable.

In the preparation of the enzyme solution the dried enzyme powder is blended with an equal amount of glycerine, the blend is suspended in water and the activity in tyrosyl units, as well as the activity expressed as total milk clotting units and available milk clotting units is determined. The value for available milk clotting units can be decreased by reversibly inactivating the enzyme solution or fractionating the enzyme with a water-miscible solvent in which the enzyme is insoluble such as acetone, dioxane or the lower alkyl alcohols.

Suitable solvents include methanol, ethanol, propanol, isopropanol, tertiary butanol, ethylene glycol, methyl Cellosolve and methyl ethyl ketone. An alternative method for decreasing the available milk clotting units is by means of salt fractionation with sodium chloride, sodium sulfate, sodium mono acid phosphate, potassium mono acid phosphate, potassium chloride, ammonium sulfate or other suitable salt. The concentration of the enzyme in solution is adjusted so that a reasonable volume of solution is injected into the animal to provide about 25 to 500 tyrosyl units/lb. of live animal weight and about 1 to 8 total milk clot units per pound of live animal weight.

Example: Crude — The enzyme is a crude dried latex which has been milled so that the particles pass an 80 mesh screen. A 75 g. quantity of this papain enzyme was blended with 75 g. of CP glycerine to form a paste and the paste was suspended in 1,500 ml. of distilled water. The crude solution was centrifuged and filtered to clarify and after Seitz filtration was employed in injecting mature sheep at levels equivalent to 0.1, 0.15, 0.20, 0.25, 0.30, 0.35, 0.70 and 1.25 ml./lb. of live weight. These levels are for a solution having 1,000 tyrosyl units/ml. and any variation from such an activity is adjusted by increasing or decreasing the amount employed in each case.

Aerated Crude — 75 g. of the crude papain was blended with 75 g. of CP glycerine and the paste taken up in 1,200 ml. of distilled water. The pH of the solution was adjusted to 7.4 with 5N aqueous sodium hydroxide solution and the final volume was adjusted to 1,500 milliliters with distilled water. The solution was clarified by centrifuging and then filtering. The filtered solution was blended in a Waring Blendor for several 1-minute periods at time intervals of 4 minutes between blending, the total blending taking place over a period of 3 hours. The blended solution was then diluted to 5 times its volume and sterilized by filtration through a bacteria retentive filter pad. This solution was injected into mature sheep at the same level as set out previously for the crude enzyme.

Peroxide Inactivated Crude — 100 g. of the crude milled papain and 100 g. of CP glycerine were mixed and the paste which was formed was taken up in 2,000 ml. of 3% hydrogen peroxide. A solution of beef liver catalase containing 200 kiel units/ml. was added at the rate of 6.65 kiel units/ml. of enzyme solution over a period of 3 1/2 hours. The solution was then clarified by filtration and the pH adjusted to 7.4 with 5N aqueous sodium hydroxide.

The final solution was sterilized by filtration through a Seitz bacteria-retentive pad and this solution was employed in injecting sheep at the levels noted previously for the crude enzyme.

Sodium Chloride Fractionated Crude — The commercial crude milled papain (150 g.) was blended to form a paste with CP glycerine (150 g.) and the paste was taken up in 2,000 ml. of distilled water. The pH of the solution was adjusted to 7.4 with the aqueous sodium hydroxide solution and held at this pH for 30 minutes. The pH of the solution was then adjusted to pH 3.5 and 685 g. of sodium chloride were added, followed by chilling of the solution to 10°C. The precipitate which was formed was collected by centrifuging and taken up in 2,260 ml. of distilled water at pH 7.4. The solution was clarified and sterilized by filtration through a Seitz bacteria-retentive pad. The sterilized solution was injected into mature sheep at levels noted previously.

Alcohol Fractionated Crude — 60 g. of commercial mill papain and 60 g. of CP glycerine were blended to form a paste and the paste was taken up in 1,200 ml. of distilled water, cooled to 0°C. The pH of the solution was adjusted to 7.4 with 5N aqueous sodium hydroxide and the solution was clarified by filtration. The enzyme was precipitated from this aqueous solution by the addition of 4,000 ml. of ethanol. This amount of ethanol provides approximately an 80% ethanol solution. The precipitate which was formed was collected by centrifuging and then dissolved in 1,000 ml. of distilled water at pH 7.4. 15 g. of sodium chloride were added and the solution sterilized by filtration through a bacteria-retentive Seitz pad. The sterilized solution was used in the injection of mature sheep, as with the preceding samples.

Acetone Fractionated Crude — 60 g. of commercial crude milled papain and 60 g. of CP glycerine were blended to form a paste and the paste was taken up in 1,200 ml. of distilled water, chilled to 10°C. The pH of the chilled solution was adjusted to 7.4 and the solution was then clarified by filtration. The enzyme is precipitated from this aqueous solution by the addition of 3,000 ml. of acetone cooled to 10°C. The precipitate was collected by centrifuging the solution and this precipitate was then dissolved in 1,000 ml. of distilled water adjusted to pH 7.4. 10 g. of sodium chloride were added and the solution sterilized by filtration through a bacteria-retentive Seitz pad. The solution was injected into mature sheep at the levels noted previously.

Sodium Sulfate Fractionated Crude — 50 g. of crude milled papain and 50 g. CP glycerine were blended to form a paste and the paste was taken up in 1,000 ml. of distilled water. The pH of the solution was adjusted to 7.4 and the solution was held at this pH for 30 minutes, at which time the pH was adjusted to 5.0. 400 g. of sodium sulfate were added and the solution was stirred until the salt dissolved. 20 g. of filter aid was added and the precipitate which was formed was collected by filtration. The precipitate was taken up in 1,000 ml. of distilled water at a pH of 7.4 and the solution was filtered to clarify and sterilize. This solution was used in injecting mature sheep, as with the foregoing samples.

Dialyzed Crude — 37.25 g. of commercial milled papain and 37.25 g. of CP glycerine were blended to form a paste and the paste was taken up in 745 ml. of distilled water at pH 7.4. The solution was introduced into Visking dialysis tubing and dialyzed against

distilled water for 48 hours. The final volume of the solution was 1,195 ml., representing a dilution of 1.6. 24 g. of sodium chloride were added and the solution adjusted to pH 7.4, followed by filtering through a bacteria-retentive Seitz pad to clarify and sterilize. This solution was injected into mature sheep at the levels noted previously.

## POST-RIGOR MORTIS ENZYME TREATMENT

Rigor mortis and post-rigor mortis toughening of meats is ascribed mainly to the formation of the actomyosin complex from actin and myosin. Subsequent tenderization involves proteolysis of the protein complexes and connective tissue in connection with ionic rearrangement to increase the degree of protein hydration.

W.O. Fraesdorf; U.S. Patent 2,961,324; November 22, 1960 has introduced an improved tenderizer composition by treating meat with a solution or aqueous dispersion of a proteolytic enzyme of animal or vegetable origin and a controlling agent comprising a somewhat partially hydrolyzed, and optionally a salt of glutamic acid such as monosodium glutamate.

Preferably the solute material is prepared in the form of mixed pulverulent materials. Substantially any active proteolytic enzyme may be used, such as trypsin, pepsin and other common enzymes of animal origin, and commonly known enzymes of vegetable origin such as papain, ficin (from figs), bromelin (from pineapple), asclepain (from milkweed latex), arachain (from peanuts), and the mixed proteases present in ripened grains. All of the above enzymes are of the protease type and active on the entire protein molecule.

Proteolytic enzymes from yeast can be effective. The preferred enzymic agent, however, is papain because of its availability, its relatively small cost, and its other distinctly advantageous properties. Moreover, a highly pure papain product is not required. The commercially available dry extracts of papaya juice containing substantial proportions of active papain are excellent.

One specific example of the composition is to prepare a dry mixture of one part of powdered commercial papain, twelve parts of a commercial pulverulent mixture of equal parts of hydrolyzed vegetable protein and animal protein, and 4 parts of monosodium glutamate. A suitable commercial protein preparation is one sold on the commercial market for making "beef bouillon." The mixture so prepared may be kept indefinitely. When meat is to be treated up to one ounce but preferably half an ounce of the mixture is dissolved in warm to hot water.

It is allowed to stand five to ten minutes and poured into a shallow pan. Slices of beef tenderloin tip, for example, of a size for broiling are placed in the shallow pan and turned over to wet them with the solution on both sides. A fork may be used to pierce the meat slices. This assists in the penetration of the solution and serves to test the character of the meat. About ten minutes treatment is usually sufficient. The beef tenderloin tip slices are then broiled, and when completed have all the tenderness and chew characteristics of the best steak cuts of the choicest beef. The treated and conditioned meat may be broiled immediately, stored for several hours in a refrigerator before cooking, or frozen and kept for several days. The solution does not have to be removed before long storage of the meat as

there is no long continuing action such as usually characterizes "tenderizers". The protein products added to the enzyme apparently have either a buffer type action, serve to modify the enzyme in some manner, or possibly form a loose chemical bond with the enzyme. It may be that some other action entirely is the cause. Proteolytic enzymes are themselves proteins, and some interaction between the enzymes and proteins in solution appears to take place.

As pointed out, hydrolyzed proteins are most effective, and a mixture of vegetable and animal origins of hydrolyzed proteins seems to produce the best results of all. While such materials as gelatin, dried powdered egg whites and the like can be used, they also are not as effective in the mixture as the mixed hydrolyzed vegetable and animal proteins. Monosodium glutamate is most effective in the mixture. It participates in controlling and attenuating the enzyme action such as to prevent the development of a pulpy product.

Glutamic acid is an amino acid, and this may explain the joint action with the hydrolyzed protein and protease which does occur. If monosodium glutamate is not used, the best results are obtained by increasing the proportion of hydrolyzed protein used. The proportion of the ingredients may, of course, be modified as contrasted with the relative quantities shown in the specific example. For one part of proteolytic enzyme on a dry basis, from 6 to 24 parts of hydrolyzed proteins may be employed, and from 2 to 6 parts of monosodium glutamate.

## UNIFORM TENDERIZATION PROCESS

One of the problems in the use of enzymes to tenderize meats is overtenderization of outer surfaces when the cut of meat is dipped in an enzyme solution, or uneven tenderization when an injection method is used. A selective process was developed by O.O. Silberstein; U.S. Patent 3,276,879; October 4, 1966; assigned to Baxter Laboratories, Inc.

The method consists of forcibly injecting into selected portions of the carcass of a freshly slaughtered domestic meat-producing animal about 1 to 4% as based on the "hot" weight which is the dressed weight of the freshly killed carcass, of a proteolytic enzyme solution having an activity of about 0.01 to 0.05 milk clotting units/g. The method involves injecting more enzyme into the less tender portions of the carcass and less enzyme into the more tender portions of the carcass.

The osmotic pressure producing material may consist of one or more different osmotic pressure producing materials, such as sodium chloride, dextrose and monosodium glutamate. Also one or more proteolytic enzymes may be used such as papain, ficin and bromelin and various animal and microbial proteases. The solid composition is dissolved in water in order to produce an approximately isotonic solution. Such approximately isotonic solutions will have a proteolytic enzyme activity of 0.01 to 0.05 milk clotting units/g. of solution. If desired, such solutions may be prepared directly and utilized as such for the tenderization of meat.

For example, a liquid meat tenderizing preparation may be prepared by producing an aqueous solution containing 26.5 to 37.5 g. of sodium chloride per gallon and sufficient proteolytic enzyme so that the proteolytic activity of the solution is 0.01 to 0.05 milk clotting

units per gram of solution. Alternatively, a meat tenderizing mixture may be prepared by mixing together 26.5 to 37.5 g. of sodium chloride and sufficient proteolytic enzyme such as papain, ficin or bromelin and various animal and microbial proteases so that upon dissolving it in one gallon of water a solution is obtained which is approximately isotonic and has a proteolytic activity of 0.01 to 0.05 milk clotting units/g. of solution.

The preferred enzyme for use is papain. The papain of commerce is derived from the latex of the tropical plant Carica papaya L. Other enzymes which may be used include bromelin, ficin and various animal and microbial proteases.

The proteolytic activity of the papain in milk clotting units is determined by the milk clotting assay method as originated by Ball and Hoover (J. Biol. Chem. 121, 773; 1937), and modified by Hinkel and Alford (Ann. N.Y. Acad. Sci. 54, 211; 1951). The enzyme preparation is dissolved in a buffered cysteine hydrochloride solution of pH 6 and is tested at 40°C. with a 20% buffered milk solution previously standardized against a standard papain preparation of 300 units/g. The time required from the addition of the enzyme until clotting of the milk begins is measured. The results are expressed in milk clotting units or MC units. A detailed description of the milk clotting assay follows.

Pipette 25.0 ml. of the milk solution, described hereinafter, into each of a series of test tubes (25 x 150 mm.) and close them with rubber stoppers. The tubes should then be placed in a constant temperature bath at 40 ± 0.5°C. and allowed to reach temperature equilibrium (15 minutes). Caution must be exercised because the assay is quite sensitive to temperature variation (e.g. changes of 1.0°C. will introduce an error of about 10% in the result).

The enzyme to be tested is dissolved in 0.035 molar cysteine solution. This solution may be prepared by dissolving 6.3 g. $Na_2HPO_4$, 14.0 g. disodium ethylenediaminetetraacetate dihydrate and 6.1 g. L(+)-cysteine hydrochloride monohydrate in 1,000 ml. of distilled water. Usually about 4 ml. of 5N sodium hydroxide are required to adjust this solution to a pH of 6.0. The approximate enzyme concentration should be about 0.5 units/ml. The enzyme solution should be prepared immediately before use; and, solutions not used for assay within 30 minutes should be discarded.

Using a volumetric pipette, withdraw exactly 2.00 ml. of the enzyme solution and discharge the contents into one of the milk-containing tubes. Stopper the tube, shake it briefly, but gently, so as to avoid the inclusion of air bubbles, and return to the constant temperature bath.

Using a stop watch (preferably one subdivided into hundredths of a minute), measure the time from the addition of the enzyme solution until clotting of the milk begins. The tube should be rolled gently back and forth in a horizontal position while in the bath. Less than one minute prior to clotting, the milk will appear to thicken somewhat and will no longer drain readily from the walls of the tube. The smooth film of milk should be watched closely from this point on. The end point is taken as the almost instantaneous appearance of a granular character in the milk film. The time required for the end point to be reached should be not less than one and not more than 5 minutes; shorter or longer periods of time will result in larger errors. The solutions employed in milk clotting assay are the following.

Dilute Buffer: Two volumes of molar acetic acid solution are mixed with one volume of molar sodium hydroxide solution. The resulting buffer solution should be about pH 4.5. Two volumes of the thus prepared concentrated buffer solution are further diluted with 15 volumes of distilled water.

Milk Solution: 100 g. of dry milk powder (e.g., Starlac brand) should be thoroughly mixed with 425 ml. of the dilute buffer solution in a blender or similar device. A trace of octyl alcohol (2-ethyl-1-hexanol) may be added to decrease foaming during the mixing operation. Filter the mixture through cheesecloth into a clean bottle. A few drops of toluene should be added as a preservative. The resulting milk solution should be allowed to stand a few hours before use. This solution will keep satisfactorily for about a week under refrigeration. Milk clotting units per gram (MC) are calculated from the following equation:

$$\text{(a)} \qquad MC = \frac{100\ mv.}{wt}$$

w = milligrams of enzyme added to milk
t = time in minutes
m = milk factor
v = volume of milk in milliliters

The milk factor, m, is established by conducting an assay with a reference standard enzyme (available upon request from Wallerstein Company of Staten Island, N.Y.) and using equation (b) to calculate the value.

$$\text{(b)} \qquad m = \frac{(MC)wt}{100\ v}$$

where (MC) represents the value of the reference standard and the values w, t and v are as indicated in equation (a). For a freshly prepared milk solution, m will have a value of about 1.7. After a week under constant refrigeration, the value of m will be about 1.4. For precise work, m should be determined each day by calibration of the milk solution with an arbitrary enzyme standard. Proper technique will allow results to be reproduced with an accuracy of about ±5.0%.

The use of hypotonic solutions results in the passage of moisture through the cell walls and into the cells of the meat where the salt concentration is higher. The net result of the use of such solutions is an adverse effect on the texture and flavor of the treated meat. The removal of the excess moisture from within the cells is time consuming and may further adversely affect the texture and flavor of the meat. On the other hand, the use of a hypertonic solution results in a dehydration of the cells because the moisture normally within the cells passes out of the cells by osmosis. The resulting dehydration of the cells adversely affects both the texture and flavor of the meat.

It is not necessary that the carcass to be tenderized be prepared in any unusual manner. However, for satisfactory results the carcasses should be treated promptly after slaughter while they are still flaccid and warm, i.e., before the effects of rigor mortis can interfere with the distribution of the enzyme preferably within 45 minutes after slaughter. When the

enzyme solution is promptly injected the distribution of the solution is far superior to that obtained by the "live injection" or any other previously known method. The injection of the enzyme solution is conducted under pressure. To insure adequate distribution the pumping pressure can, of course, vary within reasonable limits. However, extremely high pressure may cause mechanical destruction of tissue which results in local overtenderization. Generally, a pressure is employed which falls within the range of 25 to 35 psi.

The exact volume of a given enzyme solution to be injected varies with the relative tenderness of the particular anatomical section of the carcass being injected, and that of the carcass itself. With a given carcass the volume of enzyme solution to be injected varies with the relatively less tender anatomical sections receiving a larger volume of a given enzyme solution than the more tender sections. For example, a less tender anatomical section of the carcass might receive a volume of given solution approaching the 4% upper limit and a more tender anatomical section might receive a lesser volume of solution.

Alternatively, in some instances it may be considered more desirable to selectively tenderize particular anatomical sections of the carcass by injecting a more concentrated enzyme solution into a less tender section of the carcass and a like volume of a less concentrated solution into a more tender section.

Example: The carcass of a freshly slaughtered steer was split in half. One half of the carcass was treated by injecting an approximately isotonic enzyme solution into the meat through a pumping fork comprising a hollow needle holder with 8 hollow needles each 4 to 5 inches in length and provided with 8 to 10 holes distributed along the stem of the needle. The rate of pumping was controlled by a constant volume injector calibrated to deliver 105 ml. per stroke. The solution was pumped via the described apparatus into the rounds, loins, chuck and rib sections of the carcass. During the injection step precaution was exercised to insure an even distribution of the solution intramuscularly as opposed to intermuscularly.

The solution pumped into the one half of the animal had an enzyme activity of 0.021 MC units of papain per gram and contained 31.6 grams of sodium chloride per gallon of solution. A solution containing the same additive, but no enzyme was pumped in an identical manner into the control half. The amount of solution added to each was equivalent to about 1.8% of the "hot" weight of the meat.

After injection the sides were handled in accord with routine packing house procedure, i.e., they were spray-washed, shrouded and refrigerated for 48 hours. The halves of beef were then cut into quarters and subdivided to form rib steaks, rib roasts, round steaks, round roasts and the like. The various cuts of meat from both treated and control halves of beef were then prepared for consumption in a conventional manner. The results of a taste test panel indicated that the taste, odor and flavor of the control and the treated meat were identical. However, the treated meat was judged to be more tender than the control by a statiscally significant percentage of the taste panel.

The needles and their manner of placement within the carcass are especially designed to promote the intramuscular distribution of the enzyme solution. The intramuscular deposition of the enzyme solution contributes to the superior benefits obtained by the practice of

this process, whereas, the intermuscular deposition of the solution is to be avoided, because it results in the formation of pockets of solution and other undesirable distribution patterns which may diminish the effectiveness of the process. The needles preferred for use in this process are 4 to 5 inches long and are provided along three-quarters of their length with very fine holes (0.025 inch in diameter). The needles are crimped near their upper end so they can be held in place by and oriented within the needle holder. The needles are oriented within the holder to avoid an overlapping in the spray patterns produced by adjacent needles. Preferably they orient in such a way that all adjacent holes are rotated by 90°.

## PANCREATIC AND MESENTERIC LYMPH GLAND ENZYMES

B. Federics; U.S. Patent 3,183,097; May 11, 1965 has provided a method for tenderizing meat with the use of powdered pancreatic glands and mesenteric lymph glands or their mixtures. The meat tenderizing process comprises distributing throughout meat from about 0.06 to 0.75 ounce of powdered pancreatic glands or from 0.03 to 0.38 ounce of powdered mesenteric lymph glands per 100 pounds of meat, and heating the meat to an internal temperature greater than about 110°F., the meat being heated for a time sufficient to cook it. Where the meat is ham, bacon, corned beef or pastrami, the meat is cured before heating. If desired, the meat may be smoked while it is cooking.

The use of a smaller amount of glands will result in an effective process, while the use of a larger amount may impart a bitter taste to the meat. Mixtures of powdered pancreatic glands and mesenteric lymph glands may be used. Pancreatic glands are derived from animals which give beef, pork, veal or lamb. Mesenteric lymph glands are derived from animals which give beef, pork, veal or lamb. These glands are located around the casings of the bowels of these animals and serve as purifiers: all the blood of the animals passing through these glands to be purified. Mesenteric lymph glands suitable for use may be prepared by placing the glands in a glass or stainless steel container, covering the glands with acetone, changing the acetone 2 or 3 times in an hour, after about 1 hour removing the glands from the acetone, drying them, and finally pulverizing the glands to a fine powder.

While meat tenderizing commences in accordance with this process at 110°F., generally, the meat must be cooked at a higher temperature. Where trichinae must be killed, the internal cooking temperature of the meat must be greater than 137°F.

Example: This process was used for tenderizing hams. In accordance with the process, an aqueous solution was prepared consisting of about 0.5 ounce of powdered pancreatic glands (or about 0.25 ounce of powdered mesenteric lymph glands) per 2 gallons of brine. This solution was injected into the ham to 10% of its weight, by known methods such as arterial pumping.

The ham may be subjected to a fast cure or a slow cure, the latter varying in length, although 7 days is acceptable. After curing, the ham was heated to an internal temperature of 110°F., at which temperature the action of meat tenderizing commences. The ham was cooked while its internal temperature was raised to about 140°F. The hams processed with this method were more tender and had a higher yield than those not so processed.

## COLD WATER BUFFERED ENZYME

B.E. Williams; U.S. Patent 3,156,566; November 10, 1964; assigned to Chas. Pfizer & Co., Inc. found that if the proteolytic aqueous enzyme solution is held at approximately 32°F. the action of the enzyme after multiple entry injection into the warm and flaccid carcass is buffered so that very little enzymatic action takes place before the meat is chilled in the cooler and that almost 90% of the tenderizing action of the enzyme takes place during the subsequent cooking of the meat. This produces a much more uniform tenderization of the meat. It is also advantageous to hold the enzyme solution at approximately 32°F. since at this temperature the enzyme is dormant and the solution remains uniform without self-destruction of the enzyme prior to the injection.

Buffering the action of the enzyme by cold water solution prevents over tenderization; prevents mushiness of the meat; and provides a uniformity of strength of the enzyme solution before injection into the meat meeting the requirements of the Meat Inspection Division of the U.S. Department of Agriculture. More potent proteolytic enzyme is required in the cold water buffered solutions than in the warm solutions since less time is available for the proteolytic enzyme to tender the meat.

The aqueous solution of the proteolytic enzyme may be held and used at temperatures slightly below 32°F. without freezing of the solution if a blood-level or isotonic amount of salt is used in the solution and may also be held and used at temperature somewhat above 32°F. so long as the solution after injection is not raised by animal body heat to much above 55°F.

All of the proteolytic enzymes may be used but papain is preferred because it is readily available commercially in varying degrees of refinement and potency. When this enzyme is maintained in an aqueous solution at about 32°F. it is almost completely dormant. When a freshly slaughtered warm beef carcass is injected while still flaccid and before rigor mortis is completed with such a cold aqueous solution, the temperature of the injected solution is raised about 20°F. but is not above about 55°F. It is known that little tenderization of beef takes place when the beef is chilled to 55°F. or below and at temperatures in the range between 55° and 32°F. the proteolytic enzyme is virtually dormant and inactive. Below freezing no tenderization of the beef takes place and the enzyme is completely dormant.

It is apparent that even after injection into the warm animal carcass the activity of the enzyme in the aqueous solution is buffered by the temperature of the solution in the meat in the range of from 55° to 32°F. Very little tenderizing of the beef can then take place during the normal period after injection and while the carcass is being chilled in the cooler in conventional manner to the usual temperature range of approximately 35° to 42°F. Injection of the carcass with a cold aqueous solution has the additional advantage of rather chilling at least the thinner portions of the carcass so that the initial "hot" or first chill rooms can be maintained at 34°F., which is the temperature of the holding coolers, instead of from 26° to 28°F. as is conventional.

When bromelin or ficin is used as the proteolytic enzyme in place of papain, approximately one quarter as much is used since refined bromelin and ficin are rated at approximately 4 to 7 times the potency of refined papain. Combinations of the enzymes papain, bromelin and ficin may be used with a potency approximating 40 HU/g.

Example: A 600 lb. beef steer carcass was divied into two 300 lb. sides and the left side was stich pumped with an aqueous solution of papain, the solution weighing approximately 3% of the carcass weight or 2 1/2% of the boneless meat weight of the side. This aqueous solution contained approximately 1 1/2 ounces of salt and approximately 1 g. of papain having a potency of 20 HU/g. with the aqueous solution maintained at approximately 32°F. The aqueous enzyme solution was injected into the left side of the carcass at pressures approximating 35 to 100 lbs./in.$^2$ and averaging about 45 lbs./in.$^2$. The injected side and the control side were then hung in a cooler for 24 hours at from 28° to 34°F.

Steaks were then cut from the rib end of the loin both of the treated side and of the control side. These steaks were cooked and organoleptic tests showed very little difference in tenderness between them. At the end of 5 days of hanging in the cooler other steaks were removed from both the control side and the treated side and were cooked. Organoleptic tests showed that steaks from the treated side exhibited more tenderness than the steaks from the control side. The same results obtained for steaks removed from the control side and the treated side after 7 days of hanging in the cooler but the increase in tenderness of the meat of the treated side was not considered as exhibiting a commercially significant improvement in tenderness.

The same process was then carried out as in the above example utilizing a cold aqueous papain solution in which the papain was rated at 100 HU/g. and the control side and the treated side were then hung in the cooler as before. At the end of 24 hours of hanging in the cooler, steaks were removed as before and cooked. The cooked steaks from the treated side had soft spots. At the end of five days and after seven days of hanging in the cooler, the steaks removed and cooked from the treated side exhibited mushiness and over tenderization.

The same process as described in the example above was then conducted using a cold aqueous injection solution of papain at approximately 32°F. and of a potency of 40 HU/g. After 24 hours of hanging in the cooler, steaks were removed from the treated side and from the control side and were cooked. The steaks from the treated side exhibited discernibly more tenderness than the steaks from the control side. After 5 and 7 days of hanging in the cooler, steaks from the control and treated sides were cooked. Steaks from the treated side exhibited no soft spots and were voted by the taste panel as at least 20% more tender than the cooked steaks from the control side.

The process of the above example was then followed using bromelin as the proteolytic enzyme and using 1/7 the amount thereof as compared to papain. No discernible improvement in tenderness of the treated meat as compared to the control was found by organoleptic testing after hanging the meat in the cooler for from 1 to 7 days.

When bromelin was used in the cold aqueous enzyme solution approximating 1/4 the amount of papain, the taste panel found at least 20% improved tenderness in the treated meat as compared to the control. It can be concluded that the cold water buffering of the proteolytic enzymes arrests or delays the action of the enzyme between the time of injecting the carcass and the cooling of the carcass to temperatures at which the enzyme is relatively inactive while "unbuffering" or releasing the enzyme when activity is desired when the meat is raised in temperature prior to and in preparation for cooking and during the cooking of the meat.

## PAPAIN AND BROMELIN COMBINATIONS

A special combination of papain and bromelin is used in a saline solution for tenderization of meat according to a process by R.B. Sleeth and J.F. Campbell; U.S. Patent 3,166,423; January 19, 1965; assigned to Armour and Company.

Papain is effective in the tenderizing of normal protein, such as muscle, while bromelin is particularly effective in the treatment of collagenous tissues, such as muscle sheaths, tendons, etc. Commercial preparation of papain has about twice the proteolytic activity of bromelin on a unit weight basis. By providing these enzymes in a warm solution (preferably about 100°F.) and in proportions which will be described, the meat cut is uniformly tenderized and the activity of the enzymes is essentially used up in the treating period.

The meat cut is injected with a saline solution containing about 0.0060 to 0.0620% of enzymes by weight based on the solution weight, the enzymes consisting of bromelin and papain in balanced relation as to activity and the solution having a temperature of about 100°F. The solution is discharged into the meat at different levels to obtain effective distribution of the enzyme through the meat and may be held for a while and then placed in a freezer for the usual freezing operation.

Any suitable means for injecting the solution into the meat may be used. Hollow needles having spaced openings through the length of the needle are used so that when the needle is injected into the meat body, the solution will be discharged at spaced points along the length of the needle. For rapid injection, a large number of needles may be used, and these in turn fed from a manifold so that a substantial portion of the meat can be injected at one time. By way of specific example, a manifold may be provided with 4 rows of stainless steel needles containing three needles per row on two-inch centers, the needles being alternately spaced so that the distance between any two needles is about 1 1/4 inches. The needles may be 3 3/4 inches in length with 8 apertures spaced one inch apart, the first aperture being 1/4 inch from the tip end.

Sodium chloride is used at 8 to 12% based on the weight of the solution, best results being obtained at about 9%. This amount of salt does not interfere with the taste of the product and it is found to be very effective in preventing drip and shrinkage.

Best results have been obtained when the bromelin is present in about one-half the amount by weight of the amount of papain, with the bromelin percentage by weight being from 0.0040 to 0.0410 and the papain percentage being 0.0020 to 0.0210 by weight based on the solution. Best results have been obtained when the percentage of bromelin is 0.0056 to 0.0392 and the percentage of papain is 0.0028 to 0.0196. In each solution, the bromelin in general is about twice that of the papain. Within the limits suggested, further combinations of the bromelin and papain can be used for the treatment of specific meats which vary in their requirement for tenderization.

It is best to use a relatively small amount of bromelin and papain in the treatment of meat known as U.S. Good, while a larger amount is used for the treatment of U.S. Standard, and a still larger amount is used for the treatment of U.S. Commercial, while an even larger amount is used for treatment of Utility and Canner-Cutter meats including loins, rib eyes

and rounds. The solution, which is warmed to 100°F. is injected into cold cuts which may have a temperature of 35° to 40°F. or in freshly-slaughtered carcasses or cuts having a temperature of around 104° or 105°F.

The pressures will vary depending mainly on the size or weight of the meat body being injected and may vary from 75 to 175 psi. For example, for a small cut of beef weighing 10 lbs., the solution would be injected under a pressure of about 75 lbs., while in the treatment of a standard round weighing 100 lbs., the pressure may be as high as 175 lbs. or higher.

Example: 5 pairs of U.S. Good (Armour 32) boneless strip loins, chilled to 40°F. were injected with a solution containing the following where percentages are based on the weight of the solution.

| | Percent |
|---|---|
| Salt | 9.0 |
| Bromelin | 0.0056 |
| Papain | 0.0028 |
| Water | 91.0 |

The right loin of each pair was injected with the solution as described above, and each left loin served as the control. 3% of the solution based on the weight of the meat was injected into the meat at a pressure of 100 lbs., the solution being at a temperature of about 100°F.

In addition to the above product, 4 GAQ (Australian) frozen boneless strip loins were cut into half so that the anterior ends of loins 1 and 3 and the posterior ends of loins 2 and 4 were injected with the solution as described above and a solution having twice the amount of bromelin and papain respectively after defrosting. The remaining half of each loin served as the control. After tenderization, these loins were subjected to a panel for evaluating the tenderized steaks.

The enzyme-treated U.S. Good strip loins were all significantly improved with respect to tenderness. Not only was the acceptability enhanced to a tenderness equivalent to that associated with at least top Choice or higher, but equally important, is the evenness and uniformity of tenderization between different animals within the same grade without any over-tenderization. Flavor was improved in all samples with the exception of one where there wasn't any difference between the treated and control.

## PYROPHOSPHATES AND PROTEOLYTIC ENZYMES

Basic pyrophosphate salts can be used with enzymes to increase the tenderness of meats according to a process by S.L. Komarik; U.S. Patent 3,147,123; September 1, 1964; assigned to The Griffith Laboratories, Inc.

The basic pyrophosphate salt can be applied dry or in liquid form to the meat. In dry form, the pyrophosphate may be applied directly to the meat by dusting or passing the meat over and in contact with a pan of the pyrophosphate in powder form. To achieve thorough

distribution of the pyrophosphate over the meat it may be first combined with a carrier or diluent such as sugar or salt. The most advisable way to apply the basic pyrophosphate to meat is by dipping the meat in an aqueous solution of the basic pyrophosphate. However, the aqueous pyrophosphate solution also may be sprinkled or sprayed onto the meat. Other materials such as salt or flavoring agents, like sodium glutamate, may be included with the phosphates and applied to the meat simultaneously. The preferred amount of pyrophosphate to be added, is about 4 ounces per 100 lbs. of meat.

The tenderizing action of the basic pyrophosphate on meat is considerably slower than proteolytic enzymes, and it is generally advisable to hold the meat cuts so treated for at least 6 hours before cooking the meat. The phosphate treated meats need not be frozen or cooked immediately as there is essentially no danger of overtenderization. This is an obvious advantage to the meat packer, both as to preparing and packing the meat as well as in distributing it without freezing.

It is adequate to merely keep the meat at regular refrigerator temperatures without further precautions. At such temperatures it may be kept as long as 5 to 7 days without adverse effects. To obtain the osomotic action of the basic pyrophosphate on the meat and permit it to penetrate to the center of the meat and effect tenderization throughout, it is advisable not to cook the meat for at least 6 hours, and advisably up to 16 or more hours after treatment. Thicker steaks or meat cuts one inch or over obtain good tenderization in 16 to 24 hours in a holding cooler at 38° to 42°F.

It has also been found that meat can be treated with a basic pyrophosphate and a very small but effective amount of a meat tenderizing proteolytic enzyme simultaneously to increase the meat tenderness. Meat so treated can be stored under normal refrigeration without any undue precautions and tenderness is obtained without the development of mushiness. The pyrophosphate through the osmotic action it creates carries the proteolytic enzyme throughout the meat and thus thoroughly distributes it so that enzymatic action is not restricted to the meat surface.

The amount of enzyme required to effect the tenderization in combination with the basic pyrophosphate is different for each enzyme, its proteolytic activity (i.e., purity or concentration) and the kind and grade of meat being treated. The amount of proteolytic enzyme, and particularly papain, used need not usually be over 2.00 g. nor less than 0.025 g./100 pounds of meat. Advisably, 0.30 to 100 g. of enzyme per 100 pounds of meat is used. Meat cuts treated with both the pyrophosphate and enzyme can be kept in a cooler (temperature 38° to 42°F.) for from 1 to 7 days without any damage to the meat if such amounts of enzyme are used.

Example: The meat used is canner grade rib eye sliced to 1" thickness. As a control, nothing was added. The treated portion had added: (a) a liquid solution of 50% by weight tetrapotassium pyrophosphate and 50% by weight water; (b) tetrasodium pyrophosphate, dry. For the control, 6 slices weighing 885 g. were wrapped individually in cellophane and put in a cooler at 34° to 36°F. For the liquid treated material, 6 slices weighing 885 g. were dipped in the solution, wrapped individually in cellophane and put in cooler at 34° to 36°F. The steaks were left in liquid for 6 minutes. For the dry treated portion, 6 slices weighing 885 g. were dusted with powdered tetrasodium pyrophosphate, wrapped individually in cellophane

and put in cooler at 34° to 36°F. After 24 hours in the cooler, two steaks from each lot were placed on the same broiling plate and were broiled for 7 1/2 minutes on each side, broiling temperature 350°F. Taste evaluation: Control, tough; liquid treated, tender; dry treated, not as tender as liquid treated but much more tender than control. Flavor evaluation: Liquid treated steaks had slight metallic taste; dry treated had better flavor than control. Analysis for added phosphates: liquid treated, 1.18% and dry treated, 0.30%. Higher percent of added phosphates in the liquid treated steaks is responsible for the metallic flavor.

## SODIUM CHLORIDE AND PYROPHOSPHATES IN ENZYMES

Use of low levels of proteolytic enzymes for uniform tenderization throughout the meat is achieved by a special combination of sodium chloride and alkali metal pyrophosphate in a process by W. Delaney; U.S. Patent 3,188,213; June 8, 1965; assigned to Kadison Laboratories, Inc.

Example:

| | |
|---|---|
| Sodium chloride | 93 lbs. 12 oz. |
| Tetrasodium pyrophosphate | 3 lbs. 12 oz. |
| Monosodium phosphate | 2 lbs. 8 oz. |
| Papain | 8.75 oz. |

The composition of the above example was utilized on the basis of 1 lb., 9 1/2 oz. to 1 gallon of water. The monosodium phosphate was used essentially to aid in effecting solution of the tetrasodium pyrophosphate. The level of the papain in 100 gal. of the finished tenderizing composition, utilizing the composition of the above example, is approximately 12 to 13 oz. Fresh meat of utility grade was dipped into the tenderizing solution made as described above in the example, and maintained in the solution, at a temperature of about 45°F., for a period of 45 seconds. The fresh meat, upon removal from the tenderizing solution, was found to have been very effectively tenderized.

## PROCESS FOR UP-GRADING MEAT QUALITY

In determing the palatability and grade of meat, one must consider various aspects of the meat product. These considerations can be grouped as follows: fat content, moisture content, shape, tenderness and flavor. The known processes for artificially treating cuts of meat which are below the standard of the usually accepted prime cuts have generally been directed to the improvement of only one of these particular conditions in a single operation.

The processes previously in use for providing added fat to lean meat generally fall into two categories; namely, larding and marbling. Larding is a relatively old process where a substantial amount of fat is placed at various intervals within the meat in order to improve the cooking qualities of the meat and add flavoring during the cooking process. The larding technique has only proved to be successful to a certain degree in that the resulting effect of this insertion is limited to the general area of the meat in which the fat is placed and does not permeate the entire cut of meat. Accordingly, the undesirable features of having

intermediate portions of tough unflavored meat is a definite drawback. Marbling, which is the injection of fat throughout the meat to not only disperse the fat properly but also to create the effect of prime beef, has proven to be more satisfactory. The presently known process for artificial marbling is by means of injecting a liquefied fat into the main arterial trunk of a freshly slaughtered animal whereby the fat will follow the arteries and capillaries thus giving a marbleized texture to the meat.

The main disadvantage of this technique is that it requires very particular and careful slaughtering of the animal and an injection of the fat at a specified time, such as before rigor mortis. Accordingly, this technique adds greatly to the expense of the preparation of the animal and must be reflected in the final price of the meat to the consumer.

Most of the recent attention given to artificial treatment of meat has been in the tenderizing field. The natural means of tenderizing meat is through the aging process where bacterial action breaks down the fibers of the meat over a period of time. The two major processes of artificially tenderizing meat are the dip method and the use of a powder on the meat itself. In both of these processes the active ingredient used for tenderizing is a proteolytic enzyme which acts on the meat fibers.

When a powder is to be used for tenderizing the meat, it is generally intended for use by the consumer and is applied just prior to cooking the meat. The powder is sprinkled or brushed on the meat and allowed to stand for several hours in order to allow the ingredients to react with the meat. In the dip process, the entire piece of meat is dipped in a solution containing the enzymes and this also must be drained and allowed to sit for several hours to obtain a tenderizing action before cooking.

A single process which will generally improve inferior grades of meat is described by M.S. Baum and F.R. Moreo; U.S. Patent 3,215,534; November 2, 1965. The description of the process involves three essential considerations: The Composition, Preparation of the Composition and Preparation of the Meat.

The Composition: The composition consists of the following ingredients: (1) Clean strained rendered fat, which may be any of the known fats such as beef, lamb, pork, veal or vegetable fats. The choice of fats is the controlling factor in the flavor which is added to the meats and, accordingly, normal usage would dictate that one of the animal fats be chosen for the particular meat which is to be treated. However, a vegetable fat could be used and an artificial flavoring added to the compound in order to obtain the desired flavor.

(2) A proteolytic enzyme tenderizer in a liquid solution which is blended with the above fat. Any proteolytic enzyme tenderizer may be used but the particular qualities and reactions desired dictate a preference in the use of a particular type of tenderizer. This is a type of tenderizer that remains dormant and does not act on the fibers of the meat until the meat reaches a cooking temperature of approximately 120°F. and above, and which returns to a dormant stage either upon the removal of cooking temperatures or after a definite time where the tenderizing action is completed. In either case there will not be such a complete tenderizing action that the meat will shred and fall apart. One such tenderizer meeting the above requirements is a mixture of sodium chloride, sugar, a vegetable enzyme such as bromelin, and a flavoring and seasoning mixture consisting of monosodium glutamate, yeast, caramel and

a hydrolyzed vegetable protein. A mixture of this tenderizer contains sodium chloride, 46%; sugar, 37%; bromelin, 1% and flavoring and seasoning, 16%.

(3) The third ingredient is water, which of course should be purified in order to avoid the addition of any foreign flavoring or mineral content. This water acts as a carrier for the proteolytic enzyme in solution and when combined with the fat and maintained at a chilled state provides a blended composition having the consistency of margarine.

Preparation of the Composition: The basic requirement of the process is that the composition be prepared in such a manner that it will remain in a blended state whether frozen, chilled or in liquid form until injection thereof into the meat. The fat to be used is finely ground and placed in a stainless steel or aluminum kettle and brought to a boil. While the fat is at a high temperature, it is strained into a clean container and allowed to remain in the container until it has cooled to approximately 110°F.

A preservative is then added to the liquid fat in the proportion required to meet Government Standards. One ounce of the proteolytic enzyme mixture mentioned above is mixed with 2 1/2 lbs. of cold water that has been purified. This mixing is continued until the enzyme mixture is completely dissolved in the water and the resultant solution is allowed to stand for approximately 5 minutes.

Then, 2 1/2 lbs. of the strained, clean, rendered fat at approximately 110°F. is placed in the blender. The blender is then run at a relatively low speed for approximately 1 minute and the speed is then increased to the high blending speed, at which time the enzyme and water solution, approximately 2 1/2 lbs., is poured rapidly into the blender. The high speed blending operation is continued for approximately 30 seconds which produces a composition which is completely blended as to all ingredients and which has the consistency of damp margarine.

This composition may be retained without separation or deterioration by either placing it in a refrigerated cooler between 38° and 42°F. or it may be frozen for shipping purposes. When the composition is to be used for injection into the meat, if it is not in liquid form, it is reheated gradually to approximately 110°F. while undergoing constant agitation. This agitation and heating results in a liquefied blend which does not congeal.

Preparation of the Meat: The method of introducing the liquid solution into the meat is by the injection method. The liquefied composition is introduced into the meat through a hollow needle at rather closely spaced intervals. This type of injection is known in the meat treating art as "jab" or "stitch" pumping and will be referred to hereinafter in this manner. The pressure at which the liquefied composition is introduced into the meat will vary according to the size of the needle used and will normally be approximately 40 to 60 lbs.

The amount of the composition placed in the meat will be controlled according to limits set forth by the governing laws which vary to a certain degree in every state. Normally, the allowable limits approximate one portion of the composition to 5 equivalent portions of meat. Since the meat is cold due to chilling, the injection of the liquefied composition at an elevated temperature results in the congealing of the fat or suet at the point where it is injected into the meat and the water acts as a carrier for the proteolytic enzyme. The

pressure of injection causes a dispersion of this liquid throughout the body of the portion of the meat being treated.

## SELECTIVE ACTIVITY IN MEAT

Recently, high temperature-short time canning procedures have been devised which substantially reduce the period of time needed to sterilize a canned product. As a consequence, the time which the product is subjected to cooking is considerably reduced. This factor gives rise to a distinct problem with regard to the tenderness of the meat component of products such as beef stew, beef stroganoff, beef goulash, beef pot pie. Because of the shorter heating times, the meat is not adequately tenderized, and if the meat is not tenderized prior to canning, it will be too tough to appeal to the consumer. However, if the meat pieces are fully cooked prior to canning, there is a problem of the meat pieces breaking up during handling when the can is subjected to agitating type retorting or during high temperature-short time sterilization. Additionally, if the meat pieces are completely pretenderized, normal handling can agitate the meat sufficiently to cause the outer layer of the meat to continually shred off.

In the process of W.R. Schack and F.G. Connick; U.S. Patent 3,533,803; October 13, 1970; assigned to Swift & Company meat pieces for canned meat products have a relatively firm surface which provides sufficient rigidity to the meat pieces to withstand the various processing procedures of a high temperature-short time canning process and which also exhibit desirable tenderness characteristics. It has been found that such properties may be obtained by dispersing throughout the meat, proteolytic enzymes such as papain, bromelin, ficin, trypsin and following this treatment, deactivating the enzyme at the surface of the meat pieces and then activating the enzyme throughout the interior portions. The product thus formed has a tenderized center, but a surface of sufficient firmness to withstand processing and storage.

The method involves introducing a proteolytic enzyme into a primal meat cut by an convenient procedure, either ante- or post-slaughter of an animal. Methods suitable for post-mortem introduction of the enzyme into the meat cut are syringe injection, gas propellant injection, puncturing the surface of the meat and placing the meat cut in contact with an enzyme solution so that the enzyme will permeate into the meat, and any other convenient procedure. The amount of enzyme to be introduced may vary within a broad range depending upon the degree of tenderization desired, the type of meat being treated, the initial toughness or tenderness of the meat and other like considerations. However, it has been found that the amount of enzyme introduced into the meat should be at least about 0.001 mg. of enzyme per pound of meat.

Once the enzyme has been introduced, an amount of time is allowed to elapse in order to attain distribution of the enzyme throughout the meat. It has been determined that when the meat temperature is about 80°F. the holding time to allow for dispersion of the enzyme may be as short as about 1 hour or less, but at lower temperatures the holding time is considerably longer. For example, at a meat temperature of about 45°F., the holding time should be about 4 hours and at temperatures of between about 30° and 40°F. the holding time should be about 24 hours.

After the enzyme has been allowed to disperse throughout the meat, the meat is cut into pieces. The meat pieces may be any shape desired, such as cubes, chunks, strips, slices, slabs, etc. It has been determined that the dimensions of these cut pieces should be at least 1/4 inch per side. The cutting operation is performed when the temperature of the meat is reduced to between 26° and 32°F.

The next step in the method is to selectively deactivate the enzyme in the outer layer of the meat piece to a sufficient depth so that this outer layer will act as a protective cover for the meat piece. This deactivation can be accomplished by adjusting the temperature of the meat pieces to a temperature such that the enzyme in the outer layer of meat will be deactivated. This temperature adjustment may be made by any convenient surface heating technique such as by immersing the meat pieces in boiling water or in a gravy, or by radiation, or the like. The duration of the heating and the temperature to which the meat pieces must be subjected is variable within a broad range and is directly related to the particular enzyme being used, and also to the concentration of enzyme which has been introduced into the meat.

The generally accepted temperatures for proteolytic enzyme activity are in a range of from approximately 98° to 185°F. with varying specific optimal temperature ranges for specific enzymes, e.g., the optimal temperature range for papain activity is from 150° to 185°F., the optimal temperature range for bromelin activity is from 140° to 160°F., the optimum temperature range for ficin is from 140° to 170°F., and the optimum temperature range for trypsin activity is from 85° to 115°F. Temperatures in excess of these optimum ranges will cause the enzyme to become deactivated.

It has been found that for the meat pieces to exhibit the required physical characteristics, the protective outer layer of the meat piece in which the enzyme is deactivated without tenderizing the meat should be to a depth of at least about 1/16 inch on each side. Therefore, the temperature to which the meat pieces are subjected should be sufficient to raise the temperature of the entire outer portion of the meat pieces to a depth of at least about 1/16 inch to a degree which will deactivate the enzyme, i.e., a temperature in excess of the optimal temperature for enzyme activity.

The processed meat pieces having dispersed throughout the mass an unactivated proteolytic enzyme, the entire outer surface of each meat piece having the enzyme deactivated, may be stored and subsequently used in food formulations. Preferably, these meat pieces should be stored in a frozen condition.

Ultimately, the enzyme in the interior of the meat pieces is to be activated in order to tenderize the meat pieces. This activation of the interior enzyme may be performed by adjusting the temperature at the core of the meat pieces to the optimal temperature for the tenderizing activity of the particular enzyme. The temperature at the core of the meat piece should be held at this optimal level until sufficient tenderization has been accomplished.

As soon as the interior of the meat has been tenderized to a sufficient degree, the enzyme is deactivated as rapidly as possible without causing a deleterious effect to the surface of the meat. This deactivation may be by any conventional heating technique including microwave heating.

Alternatively, the selective deactivation of the enzyme at the surface of the meat and/or the deactivation of the enzyme at the core of the meat piece after the meat piece has been tenderized may be performed by techniques other than heating. Possible deactivation techniques are controlled treatment of the meat with a chemical inhibitor of enzyme activity such as an oxidizing agent, e.g., hydrogen peroxide, or a chemical salt, e.g., a mercury salt. Another alternative procedure for deactivating the enzyme is to adjust the pH of the meat surfaces to a level below pH 2.5 which will deactivate the enzyme. The following examples are intended to illustrate specific embodiments of the process and should not be considered to impose any limitations on the process.

Example 1: Papain (1.81 mg./lb. of meat) was added to "fresh" (utility grade) beef clods by the nitrogen gas propellent method described in U.S. Patent 3,216,826. The clods were held for 20 hours. The temperature of the meat was then adjusted to between 24° to 30°F. and the meat diced into approximately 1" cubes. These cubes were placed into a boiling water bath for 4 minutes in order to heat the surface (1/16 to 1/8) inch deep to 210°F. and also to raise the center temperature of the cube into the temperature range of 150° to 180°F.

The cubes were then removed from the boiling water and placed into 180°F. water and held there for 1 1/4 hours. After this treatment, the cubes were mixed with other particulate food materials in gravy. The mixture was heated by a rapid heating to above 210°F. and held for in excess of 15 minutes to deactivate substantially all the enzyme. The product was then chilled to eating temperature of about 130°F. Tenderness scores for the meat pieces indicated about an 8 to 9 rating on a 10 point scale, with 1 being tough and 10 mushy. The outside layer (about the outer 1/16" layer) had excellent bite.

Example 2: Bromelin (1.81 mg./lb. of meat) was added to "fresh" (utility grade) beef clods by the nitrogen gas propellent method described in U.S. Patent 3,216,826. The clods were held for 20 hours. The temperature of the meat was then adjusted to between 24° to 30°F. and the meat diced in approximately 1" cubes. The cubes were placed into a boiling water bath for 2 1/2 minutes in order to heat the surface at least 1/16" deep to about 190°F. and also to raise the center temperature of the cube up to a temperature of above 140°F. The cubes were then removed from the boiling water and placed into 160°F. water and held there for 1 1/4 hours.

After this treatment, the cubes were mixed with other particulate food materials in gravy. The mixture was heated by rapid heating to above 180°F. and held for in excess of 15 minutes to deactivate substantially all the enzyme. The product was then chilled to eating temperature of about 130°F. Tenderness scores for the meat pieces indicated about an 8 to 9 rating on a 10 point scale, with 1 being tough and 10 mushy. The outside layer (about the outer 1/16" layer) had excellent bite.

## ACTIVATION OF ENZYMES IN MEAT

M.M. Weber; U.S. Patent 3,561,976; February 9, 1971; assigned to Midwest Biochemical Corporation describes a method of improving the tenderness of meat by introducing a solution of a proteolytic enzyme activator into the vascular system of a living livestock animal. The animal is slaughtered within a period of 5 to 20 minutes after introduction of the solution

**United States Patent** **7,250,184**
**Toren** **July 31, 2007**

# Composition and method for tenderizing meat

**Abstract**

A method of tenderizing meat comprising providing an amount of meat and treating the meat with a composition comprising an enzyme mixture consisting of bromelin, ficin, papain and/or ***actinidin***.

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**Related U.S. Patent Documents**

| Application Number | Filing Date | Patent Number | Issue Date |
|---|---|---|---|
| 60603618 | Aug., 2004 | | |

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**Field of Search:** 426/56,58,63,574,59 435/212

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***Parent Case Text***

RELATED APPLICATIONS

The present invention claims priority on provisional patent application Ser. No. 60/603,618, filed on Aug. 23, 2004, entitled "Composition and Method for Tenderizing Meat".

***Claims***

What is claimed is:

1. A method of tenderizing meat, the method comprising: a) providing a suitable amount of meat; and b) treating the meat with a composition comprising an enzyme mixture; and where the enzyme mixture has at least three proteolytic enzymes wherein the enzyme mixture includes ***actinidin***.

2. The method of claim 1, wherein the enzymes are selected from the group consisting of: bromelin, ficin, and papain.

3. The method of claim 1, where the enzyme mixture consists of about 93% bromelin, about 5% ficin, about 1% papain and about 1% ***actinidin***.

4. The method of claim 3, wherein the bromelin has a milk clotting unit range between 20-1800.

5. The method of claim 3, wherein the ficin has a milk clotting unit range between 10-1200.

6. The method of claim 3, wherein the papain has a milk clotting unit range between 10-1500.

7. The method of claim 3, wherein the ***actinidin*** has a milk clotting unit range between 10-1800.

8. The method of claim 1, where the composition used to treat the meat comprises between about 1:5000 to 1:600,000 by weight of the enzyme mixture to the meat.

9. A method of tenderizing meat, the method comprising: a) providing a suitable amount of meat; and b) treating the meat with a composition comprising an enzyme mixture; and where the enzyme mixture has at least three proteolytic enzymes and one of the enzymes is bromelin with a milk clotting unit range between 20-1800 and the enzyme mixture consists of about 93% bromelin, about 5% ficin, about 1%

papain and about 1% ***actinidin***.

10. The method of claim 9, wherein the ficin has a milk clotting unit range between 10-1200.

11. The method of claim 9, wherein the papain has a milk clotting unit range between 10-1500.

12. The method of claim 9, wherein the ***actinidin*** has a milk clotting unit range between 10-1800.

13. The method of claim 9, where the composition used to treat the meat comprises between about 1:5000 to 1:600,000 by weight of the enzyme mixture to the meat.

14. A method of tenderizing meat, the method comprising: a) providing a suitable amount of meat; and b) treating the meat with a composition comprising an enzyme mixture; and where the enzyme mixture includes ***actinidin*** and at least two other enzymes selected from the group consisting of: bromelin, ficin, and papain.

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***Description***

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BACKGROUND

A large portion of commercially raised meat is limited in value because conventional preparation methods result in meat that is unacceptably tough and dry. A variety of methods have been used to tenderize naturally tough meat, such as mechanically interrupting the muscle fibers of the meat. However, none of these methods have produced a meat product that can be cooked using conventional preparation methods, and that results in a post-preparation product that is consistently tender and suitable for human consumption. Note that the word meat as used herein means any animal protein excluding liquid proteins such as blood.

Therefore, it would be useful to have a method of tenderizing meat so that the meat can be cooked using conventional methods, and that results in a post-preparation meat product that is consistently tender and suitable for human consumption.

SUMMARY

According to one embodiment, there is provided a method of tenderizing meat, the method comprising providing an amount of meat, and treating the meat with a composition comprising an enzyme mixture [illegible] about 94% bromelin having 20-1800 MCUs (Milk Clotting Units), around 5% ficin having 10-1200 MCUs and about 1% papain having 10-1500 MCUs. In another embodiment, the enzyme mixture consists of about 93% bromelin having 20-1800 MCUs, around 5% ficin having 10-1200 MCUs, about 1% papain having 10-1500 MCUs and about 1% ***actinidin*** having 10-1800 MCUs.

In another embodiment, the meat provided is an amount between about 0.1 kg and about 10,000 kg. In yet another embodiment, the meat provided is an amount between about 100 kg and 6000 kg.

In one embodiment, the ratio of weight of the enzyme composition to the total weight of the meat being treated with the composition is between about 1:5000 and about 1:600,000.

In one embodiment, a solution of the enzymes is injected that contains water and the enzyme

composition. The solution is injected at between 4% to 25% of the weight of the animal protein being injected.

In another embodiment, the method further comprises tumbling the treated meat at a pressure and rotation speed selected to more evenly distribute the enzyme mixture throughout the treated meat. In a preferred embodiment, the pressure is a near vacuum. In another preferred embodiment, the rotation speed is about between about 10 and about 15 revolutions per minute.

In a preferred embodiment, the method further comprises exposing the treated meat to a relative vacuum in a closed container, such as a polymer bag. In a particularly preferred embodiment, the relative vacuum is about -1.5 bar.

In one embodiment, the method further comprises packaging the treated meat in a commercial package. In a preferred embodiment, the method further comprises cooking the treated meat. In a particularly preferred embodiment, cooking the treated meat comprises raising the core temperature of the treated meat until its core temperature is heated to the recommended temperature range for the type of meat.

In another embodiment, the method further comprises distributing the treated meat to an intermediate wholesale or retail establishment.

## DESCRIPTION

According to one embodiment of the present invention, there is provided an enzyme mixture that can be used to treat meat to produce a product that can be cooked using conventional methods, and that results in a post-preparation product that is consistently tender. The enzyme mixture comprises three and/or four enzymes: bromelin, ficin, papain and/or ***actinidin***. ***Actinidin*** may be obtained from New Zealand Pharmaceuticals. According to another embodiment of the present invention, there is provided a method of tenderizing meat to produce a product that can be cooked by conventional methods, and that results in a post-preparation product that is consistently tender. The method comprises treating the meat with an enzyme mixture according to the present invention. The enzyme mixture and method will now be disclosed in detail.

As used in this disclosure, "consumer" refers to the individual or enterprise that cooks the treated meat for eventual human consumption, and includes an individual at home, and a cook in a restaurant or a food service enterprise, among others as will be understood by those in the art with reference to this disclosure.

As used in this disclosure, percent amounts are given in percent by weight of total weight.

In one embodiment, the present invention is an enzyme mixture that can be used to treat meat according to the present invention. The enzyme mixture comprises three and or four proteolytic enzymes, and can comprise one or more than one additional substance. Each enzyme in the enzyme mixture has a specific activation temperature and a deactivation temperature. When used to treat meat together and in the proper ratios, cooking the meat causes the enzymes to work synergistically to break down the substance of the meat and results in a post-preparation product that is consistently tender and suitable for human consumption.

In a preferred embodiment, the enzyme mixture consists of the three and/or four enzymes bromelin, ficin, papain and or ***actinidin***. In a particularly preferred embodiment, three enzymes are combined in specific ratios. Suitable enzymes can be obtained from All American Seasonings, Inc., Denver, Colo.

US. Usually bromelin, when used, is in a concentration less than 97% by weight of the enzyme mixuture.

In a preferred embodiment, the enzyme mixture consists of about 94% bromelin having 20-1800 MCUs (Milk Clotting Units), around 5% ficin having 10-1200 MCUs and about 1% papain having 10-1500 MCUs. In another embodiment, the enzyme mixture consists of about 93% bromelin having 20-1800 MCUs, around 5% ficin having 10-1200 MCUs, about 1% papain having 10-1500 MCUs and about 1% ***actinidin*** having 10-1800 MCUs.

More generally, ***actinidin*** may be used as a wildcard or substitute for any of the other three enzymes (Papain, Bromelin, and Ficin) partially or completely replacing the other three enzymes. At the enzyme it is replacing stated percentage of enzyme composition and specified MCUs.

In one embodiment, the ratio of weight of the enzyme composition to the total weight of the meat being treated with the composition is between about 1:5000 and about 1:600,000.

In one embodiment, a solution of the enzymes is injected that contains water and the enzyme composition, but may also include one or more substances as stated earlier. The solution is injected at between 4% to 25% of the weight of the animal protein being injects

In another embodiment of the present invention, there is provided a method of tenderizing meat to produce a product that can be cooked by conventional methods, and that results in a post-preparation product that is consistently tender. In summary, the method comprises at least the following two steps. First, a suitable type or grade of meat is provided. As used in this disclosure, the terms "type" and "grade" are interchangeable. Second, the suitable grade of meat is treated with an enzyme mixture according to the present invention. The suitable grade of meat provided is preferably meat that potentially would be undesirably tough after being cooked by conventional methods. The meat can be partially or completely skinned, boned or both. Additionally, waste products, such as connective tissue, or excess fat can be removed. The amount of meat provided can be any amount that can be handled by equipment available to perform the method of the present invention. For example, the amount can be between about 0.1 kg and about 10,000 kg. In a preferred embodiment, the amount is between about 100 kg and about 6000 kg.

Treatment of the meat with an enzyme mixture according to the present invention can be accomplished using a variety of methods. In a preferred embodiment, the meat is injected with a solution containing the enzyme mixture using commercially available injection equipment, such as the Fomaco Injector, Robert Reiser Co., Canton, Mass. US, though any suitable injection equipment can be used as will be understood by those in the art with reference to this disclosure. Preferably, the sites of injection are less than about 7.5 cm apart. In a particularly preferred embodiment, the meat is injected with a solution containing the composition.

In one embodiment, the ratio of weight of the enzyme composition to the total weight of the meat being treated with the composition is between about 1:5000 and about 1:600,000.

For example, meat that is to be cooked by grilling or microwaving can be injected with a solution. Similarly, meat that is to be cooked by a convention gas or an electric oven can be injected a solution of the enzymes.

In one embodiment, a solution of the enzymes is injected that contains water and the enzyme composition. The solution is injected at between 4% to 25% of the weight of the animal protein being

injected.

In another preferred embodiment, the method further comprises tumbling the treated meat at a pressure and rotation speed selected to more evenly distribute the enzyme mixture or composition throughout the treated meat. The pressure and rotation speed are chosen so as to separate the fibers of treated meat without shredding or tearing apart the fibers permanently, that is, while retaining the fibers' structural cohesiveness. In a preferred embodiment, the tumbling is performed in a near vacuum at between about 10 and about 15 revolutions per minute for between about 15 and about 30 minutes. The near vacuum combined with the rotation separates the muscle fibers of the treated meat allowing more rapid and uniform distribution of the enzymes. Preferably, the treated meat is tumbled in a finned vacuum tumbler with a central sealable chamber that can be operated at a specific pressure and rotation speed such as the Model LT30, available from Lance Industries, Allenton, Wis. US or a similar device, as will be understood by those with skill in the art with reference to this disclosure.

In another preferred embodiment, the method further comprises exposing the treated meat to a relative vacuum in a closed container. Containers are selected that can be sealed to maintain a vacuum for preserving the meat. In one embodiment, the container is a polymer bag, such as available from W. R. Grace & Co., Sioux City, Iowa. After selecting a suitable container, the treated meat is placed in the container and a vacuum is applied. In a preferred embodiment, the vacuum is a near vacuum of about -1.5 bar. The container tends to assume the shape of the meat upon application of the vacuum.

In another preferred embodiment, the method further comprises packaging the treated meat in a suitable commercial package for shipping and storage, or in a suitable commercial package for retail distribution to a consumer, or both. Packaging can include labeling as required by local laws and branding with a trademark or trade name and can include decorative wrapping for marketing purposes.

In another preferred embodiment, the method further comprises cooking the treated meat before packaging. The cooking can be done by any suitable method as will be understood by those in the art with reference to this disclosure. For example, the treated meat can be placed in an oven or in a hot water bath. Preferably, the treated meat is cooked until its core temperature heated to the recommended temperature range for the type of meat. If the treated meat is cooked before packaging, then the cooked treated meat is preferably cooled also before packaging.

The treated meat can be distributed to an intermediate wholesale or retail establishment, and thereby to a consumer, or can be distributed directly to a consumer. After distribution, the consumer cooks the treated meat using conventional methods, or if the product has been cooked prior to packaging, reheats the product if desired or consumes the product without reheating. For example, the treated meat can be removed from the packaging and container and can be barbecued, grilled, microwaved, prepared on a stove top or in an oven, or cooked using another conventional method, and the post-preparation product is consistently tender and suitable for human consumption.

Enzymes for use in the present invention may comprise wild-type or mutant enzymes. The enzymes may be isolated from their cell of origin or may be recombinantly produced using conventional methods well-known in the art. It will be understood that each of the reaction conditions (such as, e.g., concentration of enzyme, ratio of enzyme:meat, mode of contacting, pH, temperature, and time) may be varied, depending upon the source of meat and/or enzyme and the degree of tenderization that is required. It will further be understood that optimization of the reaction conditions may be achieved using routine experimentation by establishing a matrix of conditions and testing different points in the matrix.

Although the present invention has been discussed in considerable detail with reference to certain

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